rectification. A genetically encoded Eag domain fragment (amino acids 1-135) was shown to restore slow deactivation to N-truncated channels. Our present study sought to further investigate Eag domain contributions to hERG gating kinetics. We coexpressed the genetically encoded Eag domain fragment (N1-135) with hERG channels bearing a deletion of the N-terminus in Xenopus oocytes and measured current with two-electrode voltage-clamp recordings. Here we report that coexpression with the N1-135 peptide led to a reduction in relative outward current and slowed recovery from inactivation resulting in channels with properties similar to those measured in wild-type hERG. Through regulation of deactivation and inactivation gating, the Eag domain determines the physiologically critical resurgent component of hERG current via a non-covalent interaction with the channel.

626-Pos

Mutations Within the S4-S5 Linker Alter Voltage Sensor Constraints During Activation and Deactivation of Herg K^+ Channels

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hERG channel gating is associated with relatively slow voltage sensor movement that limits the rate of channel opening and closing. The mechanistic basis underlying the constraints upon sensor movement in these channels is unclear. Here, we have used voltage clamp fluorimetry (VCF) to study the effects of mutations within the S4-S5 linker on voltage sensor movement and its coupling to the pore. Mutations at G546 had two separable effects on activation and deactivation gating. Substitution of G546 with residues possessing different physico-chemical properties all (with the exception of G546C) shifted activation gating by ~30mV in the hyperpolarizing direction. With the activation shift taken into account, the time constant of ionic current activation was also accelerated. In addition, a number of G546 mutants affected deactivation gating, although the effects of different mutations varied. In the most dramatic case, the G546V mutation induced biphasic deactivation with a pronounced slow component that was voltage-independent. Deletion of the N-terminus accelerated the fast component, but the slow component remained pronounced, suggesting that the slow component was not mediated by altered interaction with the N-terminus. VCF measurements of voltage sensor movement in G546V channels revealed fast and slow components of fluorescence change associated with deactivation, suggesting that the slow component of ionic current deactivation is due to slow voltage sensor return that is uncoupled from charge movement. Taken together, these data suggest: 1) reduced flexibility of the S4-S5 linker helix reduces constraints on voltage sensor movement during activation gating; 2) normal hERG channel closing involves at least two reconfigurations of the voltage sensor that are rate-limiting for pore closure.

627-Pos

Rescue of Gating in hERG1 Potassium Channels Containing LQT2 Mutations in the N-Terminal PAS Domain

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The human *ether-a-go-go*-related gene 1 (hERG1) encodes a voltage-dependent potassium (K^+) channel which underlies the cardiac delayed-rectifier K^+ current (IK_r). The closing rate of the channel is a major determinant of the amplitude of outward current, and is regulated by an N-terminal Per-Arnt-Sim (PAS) domain. Loss of function mutations in hERG1 result in a loss of IK_r and lead to congenital Long QT Syndrome 2 (LQT2). Only a small percentage of the PAS domain LQT2 mutations have been characterized in mammalian cells, and these exhibited a variety of defects. Therefore, it remains unclear as to how LQT2 mutations located in the PAS domain disrupt hERG1 function. To address this, we have selected 12 PAS domain LQT2 mutations and, using biochemistry and electrophysiology, examined their functional properties when expressed at physiological temperatures. Our data demonstrate that channels with LQT2 mutations located in the PAS domain exhibit a spectrum of deficiencies when cultured at 37°C. Western blot analysis indicated that some mutations are trafficking-deficient, evident by detection of only the immature form of the channel; others were indistinguishable from WT hERG1, with enriched expression of both the immature and mature forms; while the remaining exhibited intermediate levels of maturation. Whole-cell patch-clamp analysis revealed that the LQT2 PAS domain mutants produce functional channels at the cell surface with perturbed deactivation kinetics. Co-expression of a genetically-encoded N-terminal peptide with these gating-deficient mutants rescued the gating-deficiency and fully restored the WT phenotype. Taken together, these data are the first to characterize purely gating-deficient hERG1 PAS domain LQT2 mutations expressed in mammalian cells, and show that a genetically-encoded N-terminal peptide is able to fully restore the WT phenotype to the channels.

628-Pos

Block of Herg by Trapped Drugs Shows a Different Dependency on Extracellular Potassium Compared to Block of Herg by Drugs That are Not Trapped

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Block of the cardiac potassium channel HERG by a number of drugs has been shown to decrease with an increase in the extracellular potassium concentration. This dependency on extracellular potassium can be explained by at least two mechanisms: 1) destabilization of the drug by the permeant ion 2) differential binding to the inactivated state. We have previously shown that block of HERG by quinidine, a drug that is not trapped after channel deactivation, correlates better with the permeant ion than with inactivation, indicating that quinidine block is destabilized by the permeant ion.[1] We show here that block of HERG by terfenadine and bepridil, drugs shown to be trapped in the channel after channel deactivation[2], is not altered with an increase in the extracellular potassium concentration. Furthermore block by both terfenadine and bepridil of the HERG mutant D540K, which opens with both depolarization and hyperpolarization, is decreased with increased extracellular potassium, similar to the effect of extracellular potassium on block of WT HERG by quinidine. In addition, the decrease in block of D540K by bepridil is less with an increase in extracellular cesium compared to an increase in extracellular potassium ($P_{Cs}/P_K = 0.33$). Finally, preliminary data indicate that block by bepridil of a number of HERG inactivation deficient mutants does not depend on extracellular potassium. Together these results suggest that the permeant ion is not able to destabilize a trapped drug but is able to destabilize a drug that is not trapped and suggest a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.

[1]Barrows et al. (2009) Channels: **3(4)** :239-248.
[2] Stork et al. (2007) *BJP***151**:1368-1376.

629-Pos

Conformational Flexibility of the hERG K+ Channel Pore Domain

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Malfunction of hERG K+ channels, due to inherited mutations or inhibition by drugs can cause long QT syndrome, which may lead to life-threatening arrhythmias. A 3-dimensional hERG structure is a prerequisite to understand the molecular basis of hERG malfunction. To achieve a consensus model we have carried out an extensive analysis of hERG models, based on different alignments of helix S5. The consensus model was validated using a combination of geometry/packing/normality validation, as well as molecular dynamics simulations and molecular docking. The model is confirmed by a recent mutation scanning experiment.1 Subsequently, the refined model was used to study the conformational flexibility of the hERG pore domain. Extensive molecular dynamics simulations revealed that the aromatic side-chains, lining the inner cavity can adopt a wide variety of conformations. Detailed knowledge of the hERG channel plasticity will be crucial to help interpreting differences in channel block of different drugs, since many drugs selectively block certain channel states.

1 Ju, P., Pages, G., Riek, R. P., Chen, P.C., Torres, A. M., Bansal, P. S., Kuyucak, S., Kuchel P. W, Vandenberg, J.I. (2009) J. Biol. Chem. 284, 1000-1008.

630-Pos

Substitution Scan of the S4-S5 Linker Region in KCNQ1 Channel: Structural Scaffold for Critical Protein Interactions

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KCNQ1 α-subunits are composed out of six transmembrane segments (S1-S6) that tetramerize into a functional channel. *In vivo*, KCNQ1 α-subunits associate with the β-subunit KCNE1 to generate the slowly activating cardiac I_{Ks} and consequently mutations in KCNQ1 are linked to the congenital LQT1 syndrome. Similar to other Kv channels, the S1-S4 segments form the voltage sensing domain that senses the membrane potential and that controls the